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Regulation of cAMP production in initial and terminal inner medullary collecting ducts

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Background. The inner medullary collecting duct (IMCD) is composed of at least two functionally and morphologically distinct segments, the initial (IMCDi) and the terminal (IMCDt) portions. However, most studies of receptor signaling have been performed on cells obtained from the entire inner medulla. The purpose of this study was to determine whether the patterns of receptor-activated cAMP accumulation were different between these segments.

Methods. We measured cAMP accumulation stimulated by vasopressin and isoproterenol, and the effect of epinephrine in freshly dissected IMCDi and IMCDt segments cultured and IMCDi and IMCDt cells in primary culture.

Results. The maximum response to vasopressin was twofold higher in fresh IMCDt versus IMCDi ($P < 0.05$), however, it increased in cultured IMCDi by 40% versus fresh cells with no change in the response in fresh versus cultured IMCDt. The maximum response to isoproterenol was small in fresh cells but increased by five- and sixfold, respectively, in cultured IMCDi and IMCDt cells. α_2-Adrenoceptor stimulation almost completely inhibited both vasopressin and isoproterenol-stimulated cAMP accumulations in fresh IMCDi and IMCDt cells, but only partially inhibited either accumulation by 34 to 49% in cultured cells.

Conclusions. (*1*) IMCDi and IMCDt cells are both subject to vasopressin and α_2- and β-adrenergic regulation of adenylyl cyclase activity; (*2*) the relative influence of β-adrenergic, α_2-adrenergic and V_2 receptors to affect cAMP accumulation is altered in primary culture versus freshly dissected IMCD segments, suggesting that caution must be exercised in the extrapolation of data from cultured IMCD cells to *in vivo* models.

The inner medullary collecting duct (IMCD) is the site of final adjustment of the urinary composition. Recent studies in rats and rabbits have demonstrated cellular and functional heterogeneity within the IMCD. Morphologically, the cells of the first 20 to 30% of the IMCD contain cells that resemble the principal cells of the inner stripe of the outer medulla and a small (~10%) population of intercalated cells [1–3]. Functionally, this segment has low basal water and urea permeabilities [4, 5]. Under the influence of vasopressin, the water but not urea permeability increases many-fold. This segment has been referred to as the initial IMCD (IMCDi) [5]. The terminal portion (IMCDt) is populated by a morphologically unique cell type, the IMCD cell [2]. This segment has somewhat higher baseline water and urea permeabilities than the IMCDi; however, stimulation with vasopressin causes both urea and water permeabilities to increase [4, 5] via distinct cAMP-dependent mechanisms [6, 7].

α_2- and β-Adrenoceptors are found throughout the rat collecting tubule. In the cortical collecting duct, α_2-adrenoceptors have been localized to principal cells where they inhibit vasopressin-stimulated cellular adenosine 3′,5′-cyclic monophosphate (cAMP) accumulation and water and Na reabsorption [reviewed in 8]. Inhibitory α_2-adrenoceptors are also expressed by cultured rat IMCD cells [9]. These receptors are of the α_{2B} subtype [10]. However, it is unknown whether functional α_2-adrenoceptors reside in both the IMCDi and IMCDt. β-Adrenoceptor stimulation increases cAMP production in intercalated cells of the cortical and outer medullary collecting ducts which in turn decreases Cl^- transport [11] and increases K^+ secretion [12]. β-Adrenoceptor stimulation has been reported to increase cAMP production in cultured IMCD cells [9] but no associated role in renal transport has been described. We have recently shown that cultured rat IMCD cells express β-adrenoceptors of the β_1 and β_2 subtypes [13]. The regional distribution of functional β-adrenoceptors has not fully been investigated within segments of the IMCD.

Despite the fact that the cells of the IMCDi and IMCDt are functionally and morphologically distinct, little is known about how these segments differ with respect to signal transduction. Most of our information on adenylyl cyclase regulation in IMCD cells comes from cultured cell models which use cells derived from both IMCDi and IMCDt segments. The purpose of this investigation was thus threefold: (*1*) to investigate the characteristics of vasopressin-stimulated cAMP accumulation in IMCDi versus IMCDt segments; (*2*) to determine whether α_2-adrenoceptors and β-adrenoceptors were present in both IMCDi

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and IMCDt segments; (*3*) to study the differences in regulation of cAMP production in cultured versus fresh IMCDi and IMCDt segments. The results revealed that despite differences in morphology and function, both the terminal and the initial portions of the IMCD are subject to β- and α_2-adrenergic regulation. However, our results indicate that care must be exercised in the interpretation of results obtained from cultured IMCD cells, since important quantitative differences in signaling were found in fresh versus cultured cells.

METHODS

Isolation of IMCDi and IMCDt cells

Inner medullae were removed in a sterile hood from anesthetized (pentobarbital Na, 50 mg/kg) male Sprague-Dawley rats. The kidneys were cut sagittaly and the upper one-fourth of the inner medulla, which contained the IMCDi segments, and the lower one-third of the inner medulla, which contained the IMCDt segments, were carefully dissected. The middle section was discarded. The cells from each region were isolated using a combination of collagenase digestion and osmotic shock as described by Sato and Dunn [14]. Isolated cells were either prepared for primary culture or were used immediately for experiments as described below.

Cell culture

Detailed methods are published elsewhere [10, 15]. Cells were seeded in collagen-coated 96-well plates, at a density of ~50,000 cells/well in 50 μl of culture medium and incubated in an atmosphere of 5% CO_2 at 37°C. The medium was a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12 medium that contained 10 mM *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (HEPES), 42 mM sodium bicarbonate, 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate, 0.1 mM MEM nonessential amino acids, 0.1 mM sodium pyruvate, 2 mM *l*-glutamine, 20 μg/liter gentamicin, and 10% fetal bovine serum. After 48 hours in culture, the serum-containing medium was replaced with a defined serum-free medium that contained a 1:1 mixture of DMEM and Ham's F-12 with 10 mM HEPES, 42 mM sodium bicarbonate, 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate, 5 μg/ml insulin, 50 nM hydrocortisone, 5 μg/ml transferrin, 5 pM triiodothyronine, and 10 nM sodium selenite. The medium was changed daily thereafter and cells were used in assays after five days in culture. The identity of the cultured cell types was confirmed using scanning electron microscopy. Virtually all of the cells observed that were isolated from the initial portion of the inner medulla possessed single central cilia, indicative of principal cells [16]. The cells cultured from the lower third of the medulla possessed an epithelial-like morphology but were not ciliated.

cAMP assay

In cultured cells, growth media were removed one hour prior to the assay and replaced with 50 μl of Hanks' balanced salt solution (GIBCO, Gaithersburg, MD, USA) that contained 0.5 mM $MgCl_2 \cdot 6H_2O$, 0.4 mM $MgSO_4 \cdot 7H_2O$, 20 mM HEPES, 1.2 mM 3-isobutyl-1-methylxanthine, 0.95 mM $CaCl_2$, and 0.05% bovine serum albumin. Freshly isolated cells were suspended in the same Hanks' solution and were aliquoted into 96-well plates. Each plate was placed in a shaking water bath at 37°C for dose-response studies. In one study, various doses of vasopressin (10^{-11} to 10^{-6} M) and isoproterenol (10^{-9} to 10^{-5} M) were added (5 wells/concentration/plate) and incubated for 10 minutes. To evaluate α_2-adrenoceptor activity, cells were stimulated with 3×10^{-7} M vasopressin in the presence or absence of various concentrations of epinephrine and in the presence of both epinephrine (10^{-5} M) and rauwolscine (10^{-5} M). *dl*-Propranolol (10^{-6} M) was added in vasopressin studies using epinephrine to prevent concomitant activation of β-adrenoceptors. In other studies, cells were stimulated with 10^{-5} M isoproterenol in the presence or absence of various concentrations of epinephrine. No propranolol was present in these latter experiments. The incubations were terminated after 10 minutes by adding 100 μl of 10% trichloroacetic acid (TCA; final concentration of 5%). TCA was removed by two extractions with H_2O-saturated ether and samples were dried at 80°C overnight, then resuspended in 50 mM sodium acetate buffer. The cAMP content was measured by radioimmunoassay using a kit (New England Nuclear, Boston, MA, USA).

Materials

The following compounds were purchased from the Sigma Chemical (St. Louis, MO, USA): (-)epinephrine bitartrate salt, *l*-isoproterenol bitartrate, *dl*-propranolol HCl, 3-isobutyl-1-methylxanthine, [Arg^8]-vasopressin Grade VI, collagenase (type II). DMEM, Ham's F-12, HEPES, penicillin G, streptomycin sulfate, gentamicin, *l*-glutamine, sodium pyruvate, nonessential amino acids, fetal bovine serum, insulin, hydrocortisone, transferrin, triiodothyronine and sodium selenite were purchased from GIBCO. Rauwolscine HCl was purchased from Accurate Chemicals (Westbury, CT, USA).

Statistics

All results were expressed as means ± SEM. Group means were compared where appropriate with the two tailed Student's *t*-test [17]. The statistical significance was evaluated at the 95% confidence level. Concentration-response data were subjected to iterative non-linear regression analysis using GraphPad Prism (version 2.01, GraphPad Software Inc.). Values for maximum response and half-maximal effective concentration (EC_{50}) agonists were calculated from the fitted regression curves [17].

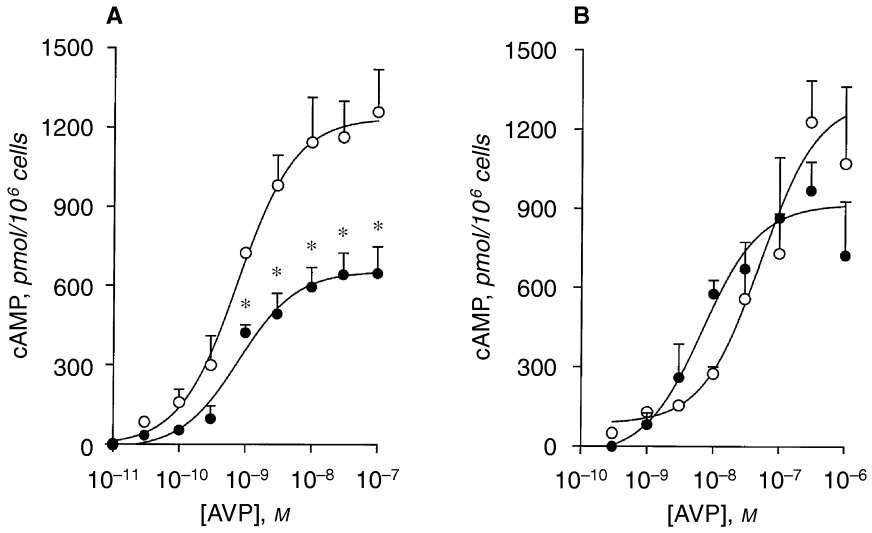

Fig. 1. Vasopressin (AVP)-stimulated cAMP accumulation in initial (IMCDi; ●) and terminal (IMCDt; ○) inner medullary collecting duct cells. (*A*) Freshly isolated cells. Each point represents the mean of three experiments ± SEM. Each experiment was performed in quintuplicate. $^*P < 0.05$ for IMCDi versus IMCDt, Students *t*-test. (*B*) Cells in primary culture. Cells were cultured in 96-well plates for five days as described in the text. Each point represents the mean of at 3 to 10 experiments ± SEM. Each experiment was performed in quintuplicate.

Table 1. Half-maximal-effective concentration (EC_{50}) values and maximum responses to modulators of adenylyl cyclase activity in initial (IMCDi) and terminal (IMCDt) IMCD cells

		Cultured cells		Fresh cells	
Agonist	Segment	EC_{50} *nM*	Max response[a]	EC_{50} *nM*	Max response
Vasopressin	IMCDi	6.8	917 ± 83	0.8	655 ± 36[b]
	IMCDt	5.1	1315 ± 164	0.7	1236 ± 62
Isoproterenol	IMCDi	75	189 ± 9	29	38.3 ± 5.6
	IMCDt	107	213 ± 11	70	30.5 ± 4.7
Epinephrine	IMCDi	24	49 ± 8%	20	94 ± 7%
	IMCDt	20	34 ± 5%	20	77 ± 6%

[a] Maximum responses to vasopressin and isoproterenol are measured in pmol cAMP/10^6 cells; max response for epinephrine is % inhibition of vasopressin (3×10^{-7} M)-stimulated cAMP accumulation. Values for maximum response were determined from non-linear regression of the concentration-response curves shown in Figures 1-3.
[b] $P < 0.05$ vs. IMCDt

RESULTS

Response to vasopressin (V_2)-receptor stimulation. In freshly isolated cells, vasopressin caused a concentration-related rise in cAMP formation (Fig. 1A). Interestingly, the maximum response to vasopressin was approximately two-fold higher in IMCDt cells versus IMCDi cells (Table 1). In cultured cells vasopressin also caused a concentration-related rise in cAMP formation (Fig. 1B). However, the maximum response of IMCDi cells in culture to vasopressin was higher compared to fresh IMCDi cells, whereas the response to vasopressin was similar in fresh and cultured IMCDt cells (compare Fig. 1 A and B). No difference was noted in the maximum response to vasopressin between cultured cells originating from IMCDi and IMCDt (Fig. 1B). The EC_{50} of vasopressin was not different between IMCDi and IMCDt cells (Table 1); however, the EC_{50} shifted rightward by 8.5 (IMCDi) and 7.2 (IMCDt) fold in cultured versus fresh cells.

Response to β-adrenoceptor stimulation. Isoproterenol elicited a small concentration-related response from freshly isolated cells (Fig. 2A). The maximum responses to isoproterenol in fresh cells were 17.1-fold (IMCDi) and 40.5-fold (IMCDt) lower than the response to vasopressin in the same segments. Maximum responses and EC_{50} values for isoproterenol were not significantly different between segments (Table 1). In cultured cells, the maximum responses to isoproterenol were 5- (IMCDi) and 7-fold (IMCDt) larger than those found in freshly isolated cells (Fig. 2B). EC_{50} values between cultured IMCDi and IMCDt cells were similar (Table 1).

Response to α_2-adrenoceptor stimulation. In freshly isolated IMCD cells, epinephrine (in the presence of propranolol) caused a nearly complete inhibition of vasopressin-stimulated (3×10^{-7} M) cAMP formation (Fig. 3A and Table 1). No significant difference was observed in the maximum response or EC_{50} values for α_2-adrenoceptor stimulation between IMCDi and IMCDt. In cultured cells there were again no differences between cell types but the maximum inhibitory response to epinephrine was blunted by 44 to 52% compared to fresh cells (Fig. 3B). The inhibitory response to epinephrine was shown to be mediated by α_2-adrenoceptors in both IMCDi and IMCDt cells in culture because the specific α_2-adrenoceptor antagonist rauwolscine completely prevented epinephrine-induced inhibition of the response to vasopressin. During these experiments, IMCDi ($N = 5$ experiments) and IMCDt ($N = 6$ experiments) were incubated with 3×10^{-7} M vasopressin, vasopressin plus epinephrine (10^{-6} M), and vasopressin plus epinephrine plus rauwolscine (10^{-5} M). Epinephrine alone significantly inhibited the response to vasopressin by 38 ± 7% (IMCDi) and 30 ± 3% (IMCDt). In the presence of rauwolscine, cAMP accumulation was 105 ± 3% (IMCDi) and 98 ± 3% (IMCDt) of the response to vasopressin alone. α_2-Adrenoceptor stimulation with

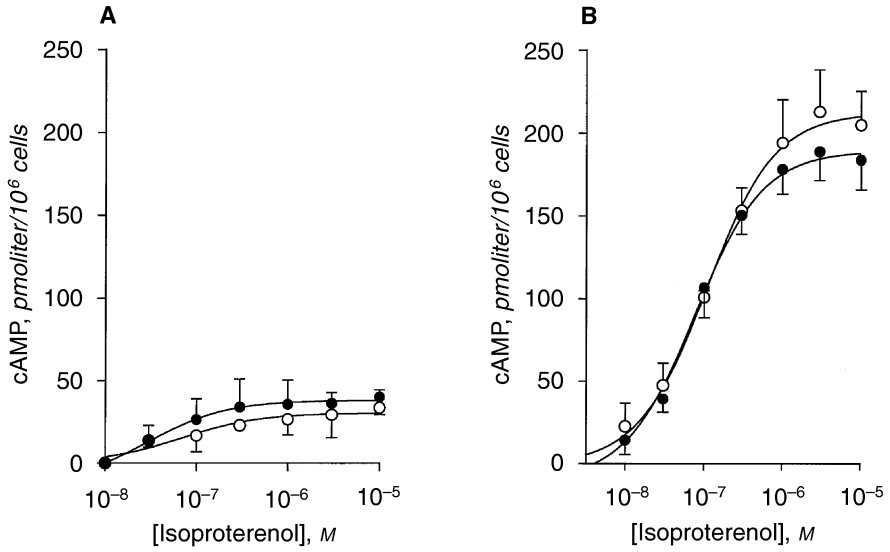

Fig. 2. Isoproterenol-stimulated cAMP accumulation in initial (IMCDi; ●) and terminal (IMCDt; ○) inner medullary collecting duct cells. (*A*) Freshly isolated cells. Each point represents the mean of three experiments ± SEM. Each experiment was performed in quintuplicate. (*B*) Cells in primary culture. Cells were cultured in plastic 96-well plates for five days as described in the text. Each point represents the mean of 3 to 10 experiments ± SEM. Each experiment was performed in quintuplicate.

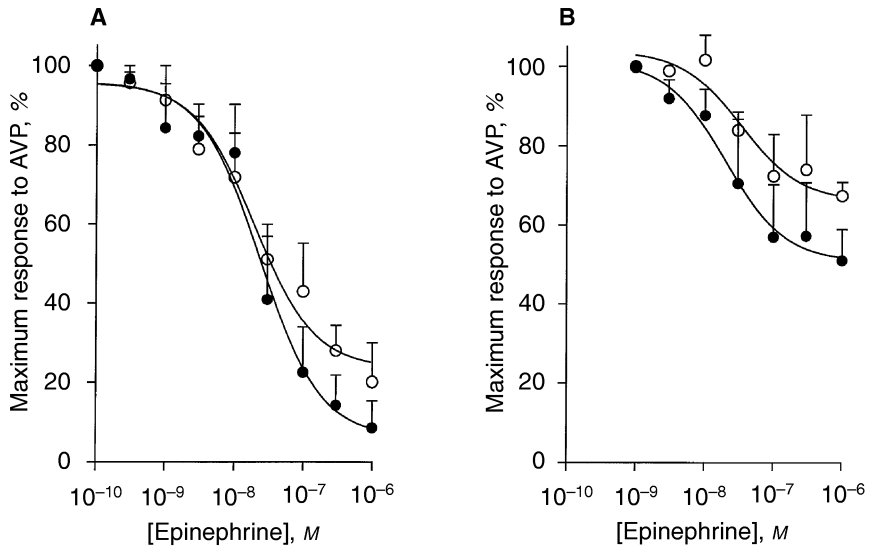

Fig. 3. Inhibition of vasopressin (AVP)-stimulated cAMP accumulation by α_2-adrenoceptor stimulation in initial (IMCDi; ●) and terminal (IMCDt; ○) inner medullary collecting duct cells. (*A*) Freshly isolated cells. Each point represents the response of 3×10^{-7} M vasopressin plus the indicated concentrations of epinephrine. The response for vasopressin alone (100%) was 648 ± 101 pmol/10^6 cells in initial IMCD segments and 1260 ± 161 pmol/10^6 cells in terminal IMCD segments. Each point represents the mean of 3 experiments ± SEM. Each experiment was performed in quintuplicate. (*B*) Cells in primary culture. Each point represents the response of 3×10^{-7} M vasopressin plus the indicated concentrations of epinephrine. The response for vasopressin alone (100%) was 970 ± 107 pmol/10^6 cells in initial IMCD segments and 1230 ± 156 pmol/10^6 cells in terminal IMCD segments. Each point represents the mean of 4 to 9 experiments ± SEM. Each experiment was performed in quintuplicate.

epinephrine also reduced the response to isoproterenol-induced cAMP formation in fresh and cultured cells (Fig. 4). The pattern of responses was similar to that seen for inhibition of vasopressin-stimulated cAMP accumulation; however, the responses in fresh cells (Fig. 4A) were quite variable due to the small baseline responses to isoproterenol (see Fig. 2A).

DISCUSSION

Most of our current knowledge of the regulation of signal transduction in the inner medulla comes from studies using primary cell culture or collecting duct cell lines. To our knowledge, studies involving primary cell culture have always used the entire inner medulla as a source for epithelial cells of IMCD origin. Signal transduction and receptor expression have been well characterized in these cells [9, 10, 14, 18, 19]. However, in the light of the recently discovered morphological and functional heterogeneity of the IMCD, it has become apparent that these cell populations must be studied separately in order to understand regulation of adenylyl cyclase by hormones and neurotransmitters in the IMCD. Further, since phenotypic alterations can occur in culture, it was necessary to quantitatively compare responses in cultured versus freshly isolated IMCDi and IMCDt cells to validate the cell culture approach. Thus, in the present study we investigated the primary known hormonal modulators of adenylyl cyclase activity in the IMCD, vasopressin and the catecholamines. Our results revealed heterogeneity of agonist-stimulated cAMP accumulation as well as important distinctions in freshly isolated versus cultured cells.

Vasopressin stimulates water transport in both IMCDi

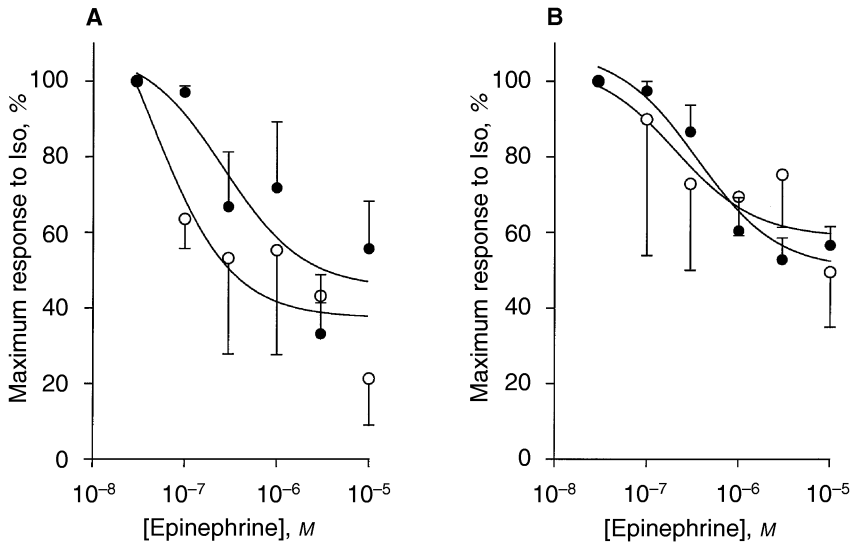

Fig. 4. Inhibition of isoproterenol-stimulated cAMP accumulation by α_2-adrenoceptor stimulation in initial (IMCDi; ●) and terminal (IMCDt; ○) inner medullary collecting duct cells. (*A*) Freshly isolated cells. Each point represents the response of 10^{-5} M isoproterenol plus the indicated concentrations of epinephrine. The response for isoproterenol alone (100%) was 40.1 ± 4.1 pmol/10^6 cells in initial IMCD segments and 33.6 ± 4.1 pmol/10^6 cells in terminal IMCD segments. (*B*) Cells in culture. The response for isoproterenol alone was 189 ± 18 pmol/10^6 cells in initial IMCD segments and 213 ± 25 pmol/10^6 cells in terminal IMCD segments. Each point represents the mean of at least three experiments ± SEM. Each experiment was performed in quintuplicate.

and IMCDt segments and urea transport in the IMCDt via cAMP-dependent mechanisms [7]. Vasopressin-stimulated cAMP formation in fresh and cultured IMCD cells is mediated by V_2 receptors [14]. In our study, cAMP accumulation in response to V_2 receptor stimulation approximately twofold greater in IMCDt versus IMCDi cells in freshly isolated preparations. Using a single vasopressin concentration, Isozaki et al [15] also showed that the cAMP response of fresh microdissected IMCDt segments was higher versus comparable IMCDi segments. Interestingly, our study showed that this difference was eliminated when the cells were cultured for five days. The EC_{50} for vasopressin was also significantly blunted in cultured versus fresh IMCDi and IMCDt cells. We attribute this attenuation to the fact that renal epithelial cells orient themselves with the basolateral (V_2 receptor-containing) membrane facing the culture substrate. Thus a higher concentration of the vasopressin peptide was needed in cultured preparations to diffuse through the confluent monolayers and stimulate the V_2 receptors. In similar studies with A6 cells, we have noted that the potency of vasopressin is higher in cells grown on permeable versus impermeable surfaces, supporting the above ideas (Jeffries and Floridia, unpublished results). The increased maximum response to vasopressin in fresh IMCDt versus IMCDi cells could be due to a higher number of vasopressin receptors, or an increase in the coupling efficiency of V_2 receptors in the IMCDt compared to IMCDi. Alternatively, vasopressin could simultaneously activate inhibitory oxytocin receptors in IMCD cells. In cultured IMCD cells it has been demonstrated that stimulation of oxytocin receptors by vasopressin can reduce cAMP formation stimulated by V_2 receptor occupation via phospholipase C-mediated crosstalk [20, 21]. It is possible that such crosstalk could play a less prominent role in the terminal versus the initial IMCD, leading to greater V_2-receptor-simulated cAMP formation in the IMCDt. Further investigation is needed to define vasopressin signaling and the relationship between V_2 and oxytocin receptors in the discrete segments of the IMCD.

Epinephrine and norepinephrine stimulate adenylyl cyclase by activating β-adrenoceptors in the collecting duct. In the cortical collecting duct, β-adrenoceptors are presumably located on intercalated cells [22], where they play a role in Cl^- reabsorption [11] and K^+ secretion [12]. β-Adrenoceptor-mediated cAMP formation has been described in IMCD cells cultured from the entire medulla [9], but the cellular location and functional role for these receptors have not been identified. Teitelbaum, Strasheim and Berl [9] tentatively identified the receptor expressed by cultured IMCD cells to be of the β_2 subtype; however, we have recently demonstrated the expression of functional β_1 and β_2-adrenoceptors by cultured rat IMCD cells using cAMP assays, radioligand binding and molecular biological techniques [13]. In the present study, we sought to confirm which segment(s) and cell types of the IMCD express β-adrenoceptors. We originally hypothesized that β-adrenoceptors would be present only in the IMCDi, since this segment possesses intercalated cells and the IMCDt does not. However, our results suggest that both the IMCDi and IMCDt express functional β-adrenoceptors, the response of which is enhanced in primary culture. We cannot be certain of the cellular identity of the cells producing β-adrenoceptor-mediated cAMP formation in the IMCDi. However, the fact that the IMCDt is sensitive to isoproterenol suggests that the IMCD cell type also produces functional β-adrenoceptors.

Our results also suggest that cell culture models may overestimate the relative magnitude of the response to β-adrenoceptor stimulation in IMCDi and IMCDt segments (Fig. 2). The maximum response to β-adrenoceptor stimulation was 17 to 19% of the maximum response to vasopressin in cultured IMCD cells; however the maximum

response to isoproterenol was only 3 to 6% of the maximum vasopressin response in fresh IMCD segments. Thus it appears that the influence of β-adrenoceptors on cAMP generation *in vivo* is likely to be small. Although cultured IMCD cells present an interesting model to study renal β-adrenoceptors, this model may not accurately reflect physiological conditions *in vivo*.

Adenylyl cyclase activation in cortical, outer and inner medullary segments of the rat collecting duct can be inhibited by the concomitant activation of α_2-adrenoceptors [8]. Stimulation of α_2-adrenoceptors causes inhibition of adenylyl cyclase and blunts the action of hormones that rely on increased cAMP levels for signaling. Studies using the isolated perfused kidney [23], micropuncture [24] and perfused segments [25] have shown that α_2-adrenoceptor agonists inhibit vasopressin-stimulated water transport in the rat collecting duct. In the cortical collecting duct, α_2-adrenoceptors are likely located on principal cells, since their activation inhibits vasopressin-induced cAMP formation [26, 27] and water and Na reabsorption. α_2-Adrenoceptors do not appear to be expressed by intercalated cells in the cortical collecting duct, since α_2-adrenoceptor agonists do not block isoproterenol-induced cAMP formation in this segment [22, 26]. α_2-Adrenoceptor stimulation inhibits the effects of vasopressin in microdissected papillary collecting duct [28, 29] and in cultured [9, 10] IMCD cells, but a distinction between IMCDi and IMCDt had not been investigated. We have recently demonstrated using radioligand binding, Northern blot analysis and cAMP accumulation that the α_2-adrenoceptor in the IMCD is of the α_{2B} subtype. In the present study we showed that functional α_2-adrenoceptors were present in both the IMCDi and IMCDt. Thus, it is likely that the principal cells of the IMCDi and the IMCD cells of the IMCDt each express α_2-adrenoceptors. Interestingly, the response to α_2-adrenoceptor activation was significantly diminished when IMCDi and IMCDt cells were placed in primary culture.

α_2-Adrenoceptor stimulation inhibits vasopressin-stimulated cAMP accumulation in both IMCDi and IMCDt cells. This suggests that these receptors may play a role in regulation of vasopressin-mediated events in the IMCDt, such as urea reabsorption. This idea is consistent with the finding reported by Maeda et al [30] that in isolated perfused IMCDt segments α_2-adrenoceptor stimulation reduced vasopressin-induced increases in urea permeability. These authors further found that α_2-adrenoceptor stimulation lowered vasopressin-stimulated cAMP accumulation in IMCDt segments, similar to our results in the present study. However, it is not yet certain whether the effects of α_2-adrenoceptor stimulation on cAMP accumulation lead to the regulation of water and urea permeability. Rouch and Kudo [31, 32] recently reported that α_2-adrenoceptor stimulation in perfused IMCD segments could reverse the effects of both vasopressin and nonhydrolyzable cAMP analogues. These authors provide evidence for a role for both prostanoids and protein kinase C in α_2-adrenoceptor-mediated inhibition of the action of vasopressin [32]. Thus, the relationship between the role of adenylyl cyclase and other pathways in the transduction of α_2-adrenoceptor-mediated effects in the IMCDi and IMCDt remains to be determined.

A primary purpose of the present study was to examine the validity of cultured IMCDi and IMCDt cells as models for hormone signaling studies. Our results showed that each of the elements (V_2 receptors, α_2- and β-adrenoceptors) of native tissue was present in cultured cells and produced qualitatively similar responses; however, the quantitative relationships among the responses were fundamentally changed. With one exception (vasopressin-stimulated cAMP formation in IMCDt cells), responses mediated via Gs, the stimulatory guanine nucleotide binding protein, were augmented in culture. Most striking was the maximum response to isoproterenol, which increased dramatically (5- to 6-fold) when either IMCDi or IMCDt cells were placed in primary culture. On the other hand, inhibition of adenylyl cyclase by α_2-adrenoceptor activation, which is mediated via Gi, the inhibitory guanine nucleotide binding protein, was markedly blunted following five days of primary culture. We have also noted a reduction in the response to α_2-adrenoceptor stimulation in cultured versus freshly isolated proximal tubule cells (unpublished observations). The mechanism(s) for these changes in phenotypic expression is unknown, but could be related to altered receptor density and/or G protein coupling. We have noted that in cells cultured from the entire medulla that the density of α_{2B}-adrenoceptors is lower than that of native tissue [10]. The mechanisms for the aforementioned phenotypic changes are unknown. Cell proliferation, growth factors, autocrine mediators, differences between *in vivo* versus *in vitro* osmolality or other changes could cause the differences we see in cultured versus fresh cells. These possibilities await investigation. However our results point out that caution must be exercised when using cell culture models to investigate signaling events that take place *in vivo*.

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